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PENNIE & EDMONDS LLP DOCKET NO. 8484-084-999

Express Mail No.: EL 451 594 794 US

08/16/00
jc904 U.S. PRO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

jc904 U.S. PRO
09/640838
08/16/00Prior application: Examiner Holleran, A.Art Unit 1642Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

This is a request for filing a ☒ continuation ☐ divisional application under 37 CFR § 1.53(b), of pending prior application no. 08/817,185 filed on September 23, 1997.

of Sinn et al.

(inventor(s) currently of record in prior application)

for CONJUGATE CONSISTING OF AN ACTIVE SUBSTANCE AND A NON-EXOGENOUS NATIVE PROTEIN

(title of invention)

1. ☒ The filing fee is calculated below:

PATENT APPLICATION FEE VALUE

| TYPE | NO. FILED | LESS | EXTRA | EXTRA RATE | FEE |
|--|-----------|------|-------|--------------|-------------|
| Total Claims | 35 | -20 | 15 | \$18.00 each | \$ 270.00 |
| Independent | 1 | -3 | 0 | \$78.00 each | \$ 0.00 |
| Basic Fee | | | | | \$ 690.00 |
| Multiple Dependency Fee If Applicable (\$260.00) | | | | | \$ 260.00 |
| Total | | | | | \$ 1,220.00 |
| 50% Reduction for Independent Inventor, Nonprofit Organization or Small Business Concern | | | | | - \$ 0.00 |
| Total Filing Fee | | | | | \$ 1,220.00 |

2. ☒ Please charge the required fee to Pennie & Edmonds LLP Deposit Account No. 16-1150. A copy of this sheet is enclosed.
3. ☐ Amend the specification by inserting before the first line the following sentence: This is a continuation of application no. filed.
- 4a. ☐ Transfer the drawings from the prior application to this application and abandon the prior application as of the filing date accorded this

application. A duplicate copy of this sheet is enclosed for filing in the prior application file.

- 4b. ☒ New formal drawings are enclosed.
- 4c. ☐ Informal drawings are enclosed.
- 5a. ☐ Priority of application no. filed on in is claimed under 35 U.S.C. §119.
- 5b. ☐ The certified copy has been filed in prior application no. , filed .
6. ☒ The prior application is assigned of record to Deutsches Krebsforschungszentrum Stiftung des Offerntlichen Rechts.
- 7a. ☒ The Power of Attorney appears in the original papers in the prior application no. 08/817,185, filed September 23,1997.
- 7b. ☐ Since the Power of Attorney does not appear in the original papers, a copy of the Power in prior application no. , filed is enclosed.
8. ☐ This application contains nucleic acid and/or amino acid sequences required to be disclosed in a Sequence Listing under 37 CFR §§1.821-1.825. It is requested that the Sequence Listing in computer readable form from prior application no., filed on be made a part of the present application as provided for by 37 C.F.R. §1.821(e). The sequences disclosed therein are the same as the sequences disclosed in this application. A copy of the paper Sequence Listing from application no. is enclosed.
9. ☐ The undersigned states, under 37 C.F.R. §1.821(f), that the content of the enclosed paper Sequence Listing from application no. is the same as the content of the computer readable form submitted in application no. .
10. ☒ Additional enclosures or instructions:
 - Copy of complete Application as originally filed;
 - a Preliminary Amendment; and
 - Please enter the Preliminary Amendment before calculating the filing fee.

Date

August 16, 2000

Respectfully submitted,

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09640836 081600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Sinn et al.

Application No.: TO BE ASSIGNED

Group Art Unit: TO BE ASSIGNED

Filed: HERewith

Examiner: TO BE ASSIGNED

For: CONJUGATE CONSISTING
OF AN ACTIVE SUBSTANCE
AND A NON-EXOGENOUS
NATIVE PROTEIN

Attorney Docket No.: 8484-084-999

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to examination of the accompanying continuation application, please enter the following amendment.

AMENDMENT

In The Specification:

Please amend the Specification as follows:

At page 1, in the first sentence following the title, after "This" insert --application is a continuation of United States Patent Application Serial Number 08/817,185, filed September 23, 1997, which--.

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At page 4, line 5, delete "flammable" and insert in its place --inflamed--.

In The Claims:

Please cancel Claims 1-3 and 6-13, without prejudice.

Please amend Claims 4 and 5 as follows:

4. (Amended) The conjugate according to claim [3] 15, [characterized in that] wherein the chemotherapeutic agent is an antibiotic.

5. (Amended) The conjugate according to claim [3] 15, [characterized in that] wherein the chemotherapeutic agent is an antimetabolite.

Please add new Claims 14-42:

--14. (New) A conjugate useful for treating a disease selected from the group consisting of tumoral, infectious, and autoimmune disease in a subject comprising:

- an active substance useful for treating said disease;
- a native human protein that is not regarded as exogenous by the subject; and
- a linker linking said active substance to said protein, wherein said linker can be cleaved intracellularly, and wherein said linker comprises an azo group.

15. (New) The conjugate according to Claim 14, wherein the active substance is selected from the group consisting of a chemotherapeutic agent and a photoactive compound.

16. (New) The conjugate according to Claim 14, wherein several active substances useful for treating said disease are linked to said protein through one or more linkers.

17. (New) The conjugate according to Claim 14, wherein the linker has the following structure:



wherein:

R is an aromatic compound, and

Y is selected from the group consisting of C(O), S(O)₂, P(O)OH and As(O)OH.

18. (New) The conjugate according to Claim 14, wherein the protein is human serum albumin.

19. (New) The conjugate according to Claim 14, wherein the conjugate comprises 4-aminophenylsulphonic acid or 4-aminophenylphosphonic acid linked to albumin, an azo group being present as linker.

20. (New) The conjugate according to Claim 14, wherein the conjugate comprises cytodine linked to albumin, a linker containing an azo group being present.

21. (New) The conjugate according to Claim 14, wherein the conjugate comprises tetracycline linked to albumin, a linker containing an azo group being present.

22. (New) A process for the preparation of the conjugate according to Claim 14, comprising binding an active substance useful for treating a disease selected from the group consisting of tumoral, infectious, and autoimmune disease to a native human protein that is not regarded as exogenous by the subject, by means of a linker containing an azo group.

23. (New) A method of treating a disease selected from the group consisting of tumoral, infectious, and autoimmune disease comprising administering a conjugate according to Claim 14 in an amount effective to ameliorate the symptoms of said disease.

24. (New) The conjugate according to Claim 15, wherein several active substances are present.

25. (New) The conjugate according to Claim 15, wherein the linker has the following structure:



wherein:

R is an aromatic compound, and

Y is a group selected from the group consisting of C(O), S(O)₂, P(O)OH and As(O)OH.

26. (New) The conjugate according to Claim 16, wherein the linker has the following structure:



wherein:

R is an aromatic compound, and

Y is a group selected from the group consisting of C(O), S(O)₂, P(O)OH and As(O)OH.

27. (New) The conjugate according to Claim 15, wherein the protein is human serum albumin.

28. (New) The conjugate according to Claim 16, wherein the protein is human serum albumin.

29. (New) The process of Claim 22, wherein said binding comprises the formation of a chemical bond selected from the group consisting of an azo group or an ester.

30. (New) The conjugate of Claim 4 wherein the antibiotic comprises a tetracycline.

31. (New) The conjugate of Claim 5 wherein the antimetabolite comprises a methotrexate.

32. (New) The conjugate of Claim 5 wherein the antimetabolite comprises a sulfonamide.

33. (New) The conjugate of Claim 5 wherein the antimetabolite comprises a nucleoside that inhibits the replication or transcription of a nucleic acid into which it is incorporated.

34. (New) The conjugate of Claim 14 wherein the active substance comprises an acid group.

35. (New) The conjugate of Claim 34 wherein the acid group is selected from the group consisting of $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, and $-\text{AsO}_3\text{H}_2$.

36. (New) The conjugate of Claim 14 wherein the active substance is selected from the group consisting of 4-aminobenzoic acid, 2-aminobenzoic acid, 4-aminophenylsulfonic acid, 2-aminophenylsulfonic acid, 4-aminophenylphosphonic acid, 2-aminophenylphosphonic acid, 4-aminophenylarsonic acid, and 2-aminophenylarsonic acid.

37. (New) The conjugate of Claim 14 wherein the active substance is selected from the group consisting of a deoxyuridine, a deoxycytidine, a cytosine arabinoside, a 5-fluorouracil, a 5-fluorodeoxyuridine, and an azidothymidine.

38. (New) The conjugate of Claim 15 wherein the photoactive substance comprises a porphyrine.

39. (New) The conjugate of Claim 15 wherein the photoactive compound is selected from the group consisting of a chlorine and a bacteriochlorine.

40. (New) The conjugate of Claim 14, wherein the native human protein comprises a transferrin.

41. (New) The conjugate of Claim 17, 25 or 26, wherein the aromatic group comprises a phenylene.

42. (New) The conjugate of Claim 17, 25 or 26, wherein the aromatic group comprises a derivative of phenylene.

REMARKS

Applicants have amended the Specification to indicate that the instant application is a continuation of United States Patent Application Serial Number 08/817,185. This amendment is made pursuant to 35 U.S.C. § 120. Applicants have also corrected a typographical error in the Specification. Neither amendment adds new matter. Accordingly, Applicants respectfully request that these amendments be entered.

With this amendment Claims 4, 5 and 14-42 are pending. The claims have been amended, without prejudice, for the purpose of more clearly defining what Applicants regard as the invention. The amendments are fully supported by the Specification and the originally filed claims of the present application. The amended claims conform to the allowed claims of United States Patent Application Serial Number 08/817,185, the parent application of the instant application. Therefore, entry of the amendments under 37 C.F.R. § 1.111 is respectfully requested.

Claims 1-3 and 6-13 have been canceled, without prejudice. Applicants expressly reserve the right to prosecute the subject matter of the canceled claims in one or more timely filed divisional, continuation or continuation-in-part applications.

CONCLUSION

In view of the above amendment and remarks, the subject application is believed to be in good and proper order for allowance. Early notification to this effect is earnestly solicited.

No fee is believed due in connection with this submission. However, the Commissioner is authorized to charge any required fee or credit any overpayment to Pennie & Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

Date August 16, 2000

Laura A. Coruzzi / By: *[Signature]*
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Enclosure

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0040330.001000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**CONJUGATE CONSISTING OF AN ACTIVE SUBSTANCE AND A
NON-EXOGENEOUS NATIVE PROTEIN**

This is a national phase filing of the Application No. PCT/DE95/01323, which was filed with the Patent Corporation Treaty on September 22, 1995, and is entitled to priority of the German Patent Application P 44 33 890.2, filed August 1, 1994.

I. FIELD OF THE INVENTION

This invention relates to a conjugate consisting of an active substance and a native protein which is not regarded as exogenous, a process for the preparation of such a conjugate as well as its use.

II. BACKGROUND OF THE INVENTION

For a long time there has been a great demand to transport in well-calculated fashion pharmaceutical preparations to certain sites within the body where they are allowed to display their activity. The former has been achieved by an above conjugate. *See*, DE - 41 22 210. It serves for concentrating a tumor-active compound in the tumor.

Surprisingly, it has now turned out that an above conjugate is also highly active when an intracellularly cleavable linker is present between the active substance and the native protein which is not regarded as exogenous.

Such a conjugate represents the subject matter of the present invention.

III. SUMMARY OF THE INVENTION

This invention relates to a conjugate which consists of an active substance and a native protein which is not regarded as exogenous and distinguishes itself in that an intracellularly cleavable linker is present between the active substance and the protein.

In addition, this invention concerns a process for the preparation of such a conjugate and its use.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the binding of 4-aminophenylsulfonic acid or 4-aminophenylphosphonic acid to albumin, an azo group being present as linker.

FIGURE 2 shows the binding of cytidine to albumin, a linker containing an azo group being present, and the release of aminocytidine.

FIGURE 3 shows the binding of tetracycline to albumin, a linker containing an azo group being present.

FIGURE 4 shows the growth inhibition of tumor cells by administration of conjugates according to the invention.

V. DETAILED DESCRIPTION OF THE INVENTION

The above expression "active substance" comprises compounds of any kind which can be used for treating a disease. They are, *e.g.*, compounds for treating tumoral, infectious and/or autoimmune diseases. Examples of such compounds are chemotherapeutic agents such as antibiotics, *e.g.*, tetracyclines, and antimetabolites, *e.g.*, methotrexate, sulfonamides and nucleosides which after the incorporation into a nucleic acid inhibit its replication and transcription, respectively. Preferred compounds of the above kind are those which have an acid group such as $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, or $-\text{AsO}_3\text{H}_2$. Especially preferred compounds are 4- and 2-aminobenzoic acid, 4- and 2-aminophenylsulfonic acid, 4- and 2-aminophenylphosphonic acid, 4- and 2-aminophenylarsonic acid as well as derivatives thereof. Further preferred compounds are deoxyuridine (UDR), deoxycytidine (CDE), cytosine arabinoside (AraC), 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUDR), azidothymidine (AZT).

Further examples of compounds as active substance are photoactive substances such as porphyrines, chlorines and bacteriochlorines which can be used for the photodynamic treatment.

One or several of the above compounds are present in a conjugate according to the invention. They are given as educts, which means that they are present in derivatized form in a conjugate according to the invention. *See*, Examples 1-7 and FIGURES 1-3, *infra*.

An above active substance is linked via a linker to a protein. This protein is not considered exogenous by the body. It is also available in native, *i.e.*, non-modified, form. In addition, the protein has a molecular weight (MW) of up to 90,000, preferably it is an albumin, particularly human serum albumin, or transferrin.

An above linker can be cleaved intracellularly. The expression "cell" comprises individual cells and cell aggregates. Examples of the former are endogenous cells which are not present in an aggregate, *e.g.*, blood cells and virus-infected cells, and exogenous cells, *e.g.*, microorganisms such as bacteria, fungi and protozoa. Cell aggregates comprise tissues, organs and tumors.

A person skilled in the art is familiar with a linker of the above kind. He is also familiar with factors, *e.g.*, enzymes, which cause the cleavage of certain chemical bonds in cells. Thus, he can construct further linkers which can be cleaved intracellularly. Such a linker favorably comprises an azo group, which is preferably cleaved. The following structure of the linker is especially favorable: wherein



R is an organic group, preferably an aromatic one, and especially preferably phenylene or a derivative thereof, and

Y is a group selected from C(O), S(O)₂, P(O)OH and As(O)OH.

The above structure of a preferred linker corresponds to that which the linker has in a conjugate according to the invention. Furthermore, at least when R is phenylene or a derivative thereof, the structure comprises an active compound which is especially suited for treating tumoral, infectious and autoimmune diseases. Having cleaved the linker and optionally degraded the protein still linked to the linker, the compound can display its full activity. *See*, Examples 3 to 7 and FIGURES 2 and 3, *infra*.

Preferred conjugates of the present invention are shown in FIGURES 1 to 3.

A process for the preparation of an above conjugate is also provided according to the invention. In such a process, conventional reactions occurring in chemistry such as diazotizing of an amino group and activation of an acid group, are used individually or in combination. Reference is made to the preparation of the conjugates in Examples 1 to 7 and FIGURES 1 to 3.

Conjugates according to the invention distinguish themselves in that they concentrate active substances in well-calculated fashion in certain cells of the body and allow them to fully display their activity. This is achieved by a combination consisting of a protein, *e.g.*, albumin, and an intracellularly cleavable linker. Certain cells in the body, particularly tumor cells, cells of flammable tissues and microorganisms, preferably absorb albumin and because of their enzymes cleave the linker-active substance conjugate, so that the active substance and substances, respectively, is (are) released and can fully display its (their) activity.

Thus, conjugates according to the invention are suited in the best possible manner for therapeutic purposes, particularly for treating tumoral, infectious and autoimmune diseases.

In addition, labelings (*e.g.*, radioactive labelings) may exist in conjugates according to the invention, so that the conjugates can also be used for diagnostic purposes and therapy control, optionally simultaneously for treatment.

The below examples explain the invention in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

VI. EXAMPLES

A. Example 1: Preparation Of A Conjugate According To The Invention Consisting Of Human Serum Albumin And 4-Aminophenylsulfonic Acid, An Azo Group Being Present As Linker

The preparation of the conjugate and its structure are shown in FIGURE 1.

1. Diazotization of 4-aminophenylsulfonic acid:

4-Aminophenylsulfonic acid (173 mg, 1 mmole) was dissolved in 5 ml 2 N HCl. The solution was cooled in an ice bath, and 600 μ l of an ice-cooled 2.5 M NaNO₂ solution (1.5 mmoles) were added in portions of 0.1 ml each with constant stirring. After about 10 min, the excess of nitrite was eliminated by the addition of urea. 4-Diazoniumphenylsulfonic acid (4-DAPS) was obtained.

2. Linkage of 4-DAPS to human serum albumin (HSA):

The 4-DAPS solution obtained under item 1. was slowly added to a solution of 2 g HSA in 30 ml of 0.17 M Bic in a molar ratio of 1:1 with pH control and constant stirring, so that the pH value was constantly above 7.5. During the addition of 4-DAPS already, the solution started dyeing red, the color constantly increasing with proceeding reaction time. Contaminations such as excess urea or salts were separated by ultrafiltration via a YM 30 membrane in an Amicon pressure filtration cell. A conjugate consisting of 4-aminophenylsulfonic acid and HSA was obtained, an azo group being present as linker.

The purity of the conjugate according to the invention was checked by means of HPLC (precolumn: Zorbax Diol 20 μ (50x4 mm), column 1: Zorbax GF 450, column 2: Zorbax GF 250, eluent: 0.2 M Na citrate, pH 7.5, flow 1 ml/min).

B. Example 2: Preparation Of A Conjugate According To The Invention Consisting Of Human Serum Albumin And 4-Aminophenylphosphonic Acid, An Azo Group Being Present As Linker

The preparation of the conjugate and its structure are shown in FIGURE 1.

The preparation was carried out as described in Example 1, 4-aminophenylphosphonic acid having been used in place of 4-aminophenylsulfonic acid.

C. Example 3: Preparation Of A Conjugate According To The Invention Which Consists Of Cytidine, A Linker Containing An Azo Group And Human Serum Albumin (Cytidine-4-DAPS-HSA)

The preparation of the conjugate and its structure are shown in FIGURE 2.

4-DAPS was prepared as described in Example 1.

1. Linkage of 4-DAPS to cytidine:

2.6 mmoles of cytidine (about 600 mg) were dissolved in 6 ml 2 N NaOH, and the 4-DAPS solution was added in portions (1 ml each) with stirring. The initially colorless cytidine solution adopts a more and more intense red color during the addition of 4-DAPS already. After completion of the reaction, the deep-red solution was adjusted with 1 N HCl to a pH value of about 2 and then lyophilized. The dry residue obtained after the lyophilization is subsequently dissolved in a mixture consisting of 8 ml of methanol and 2 ml of DMF and separated from insoluble sediment by filtration. 5(4-diazophenylsulfonic acid) cytidine (5(4-DAPS)cytidine) was obtained.

The purity of the product was checked by means of thin-layer chromatography (plates with fluorescence indicator, eluent: Etac/MeOH 1/1).

2. Activation of 5(4-DAPS) cytidine to form the corresponding HSI ester:

An aliquot of the solution of 5(4-DAPS) cytidine was admixed in the same solvent (4 parts of methanol and 1 part of DMF) with two times the molar amount of dicyclohexylcarbodiimide (DCC) and 7 to 10 times the molar amount of N-hydroxysuccinimide (HSI). After a reaction time of about 1 h, the activation of the 5(4-DAPS) cytidine into the corresponding HSI ester is terminated. It can be used directly for linkage to HSA.

3. Linkage of the HSI ester of 5(4-DAPS) cytidine to HSA:

The HSI ester of 5(4-DAPS) cytidine was slowly added to a solution of 2 g HSA in 30 ml 0.17 M Bic with constant stirring. DCC already precipitates during he addition of the HSI ester of 5(4-DAPS) cytidine. The turbid matter of DCC and DC

urea was separated by means of filtration. Other contaminations such as methanol, DMF and HSI were then separated via a YM 30 membrane in an Amicon pressure filtration cell. Cytidine-4-DAPS-HSA was obtained.

The purity of the conjugate according to the invention was checked by means of HPLC. *See*, Example 1, *infra*.

D. Example 4: Preparation Of A Conjugate According To The Invention Consisting Of UDR, A Linker Containing An Azo Group And Human Serum Albumin (UDR-4-DAPS-HSA)

The conjugate according to the invention was prepared as described in Example 3, UDR having been used in place of cytidine. UDR-4-DAPS-HSA was obtained.

E. Example 5: Preparation Of A Conjugate According To The Invention Consisting Of AraC, A Linker Containing An Azo Group And Human Serum Albumin (AraC-4-DAPS-HSA)

The conjugate according to the invention was prepared as described in Example 3, AraC having been used in place of cytidine. AraC-4-DAPS-HSA was obtained.

F. Example 6: Preparation Of A Conjugate According To The Invention Consisting Of CDR, A Linker Containing An Azo Group And Human Serum Albumin (CDR-4-DAPS-HSA)

The conjugate according to the invention was prepared as described in Example 3, CDR having been used in place of cytidine. CDR-4-DAPS-HSA was obtained.

G. Example 7: Preparation Of A Conjugate According To The Invention Consisting Of 7-Chlorotetra-Cycline, A Linker Containing An Azo Group And Human Serum Albumin

The preparation of the conjugate and its structure are shown in FIGURE 3.

4-DAPS was prepared as described in Example 1.

1. Linkage of 4-DAPS to 7-chlorotetracycline:

718.5 mg (1.5 mM) of 7-chlorotetracycline (MW 478.9) were dissolved in 20 ml 1 N NaOH, and the 4-DAPS solution was added in portions (1 ml each) with constant stirring. The 7-chlorotetracycline solution initially dyed yellow adopted a more and more intense red color during the addition of 4-DAPS. After a reaction time of about 24 h, the deep-red solution was adjusted to a pH value of about 2 using 1 N HCl and lyophilized. The dry residue was then dissolved in a mixture consisting of 8 ml of MeOH and 2 ml of DMF and separated from the insoluble sediment by filtration. 7-Chloro-9 (4-diazophenylsulfonic acid) tetracycline (4-DAPS-chlorotetracycline) was obtained.

2. Activation of 4-DAPS-chlorotetracycline for protein linkage:

An aliquot of the solution of the 4-DAPS-tetracycline was admixed in the same solvent (4 parts of MeOH and 1 part of DMF) - without preceding separation of the excess 7-chlorotetracycline - with two times the molar amount of DCC (based on the employed amount of phenylsulfonic acid) and 7 to 10 times the molar amount of HSI. After a reaction time of about 2 h, the activation of 4-DAPS-chlorotetracycline into the corresponding HSI ester is terminated. The resulting ester can be used directly for protein linkage.

3. Linkage of the HSI ester of 4-DAPS-chlorotetracycline to HSA:

The equimolar amount of HSI ester of 4-DAPS-chlorotetracycline is slowly added to a solution of 2 g of HSA in 30 ml 0.17 M Bic with constant stirring. The excess of DCC precipitates already during the addition of the HSI ester. The turbid matter of DCC and DC urea was separated by means of filtration prior to pressure filtration. Other contaminations such as MeOH, DMF and HSI were separated via a YM 30 membrane in an Amicon pressure filtration cell. 7-Chloro-9 (4-diazophenylsulfonic acid) tetracycline-HSA was obtained.

The purity of the resulting conjugate was determined by means of HPLC. *See*, Example 1, *infra*.

H. Example 8: Preparation Of A Conjugate Consisting Of Tetracycline, A Linker Containing An Azo Group And Human Serum Albumin

The conjugate was prepared as described in Example 7, tetracycline having been used in place of 7-chlorotetracycline. The structure of the conjugate is shown in FIGURE 3.

I. Example 9: Growth Inhibition Of Tumor Cells By Administration Of Conjugates According To The Invention

The conjugates UDR-4-DAPS-HSA (*see*, Example 4, *supra*), AraC-4-DAPS-HSA (*see*, Example 5, *supra*) and CDR-4-DAPS-HSA (*see*, Example 6, *supra*) as well as HSA alone as control were incubated in each case with Walker 256 cells under conventional conditions. The number of cells per ml was determined after 24, 48 and 72 h, respectively.

As follows from FIGURE 4, each of the conjugates according to the invention reduces the proliferation of tumor cells as compared to the control.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

CLAIMS

WHAT IS CLAIMED IS:

1. A conjugate consisting of an active substance and a native protein which is not regarded as exogenous, characterized in that an intracellularly cleavable linker is present between the active substance and the protein.
2. The conjugate according to claim 1, characterized in that the active substance is a compound usable for treating tumoral, infectious and/or autoimmune diseases.
3. The conjugate according to claim 2, characterized in that the active substance is a chemotherapeutic agent and/or a photoactive compound.
4. The conjugate according to claim 3, characterized in that the chemotherapeutic agent is an antibiotic.
5. The conjugate according to claim 3, characterized in that the chemotherapeutic agent is an antimetabolite.
6. The conjugate according to any one of claims 1 to 5, characterized in that several active substances are present.
7. The conjugate according to any one of claims 1 to 6, characterized in that the linker comprises an azo group.
8. The conjugate according to claim 7, characterized in that the linker has the following structure:



wherein:

R is an aromatic compound, and

Y is a group selected from C(O), S(O)₂, P(O)OH and As(O)H.

9. The conjugate according to any one of claims 1 to 8, characterized in that the protein is albumin.

10. The conjugate according to claim 1, namely the conjugate of FIGURE 1.

11. The conjugate according to claim 1, namely the conjugate of FIGURE 2.

12. The conjugate according to claim 1, namely the conjugate of FIGURE 3.

13. A process for the preparation of the conjugate according to claim 7, characterized in that the binding of the active substance via the linker to the protein comprises a diazotization of amino groups and/or an activation of acid groups.

14. Use of the conjugate according to any one of claims 1 to 12 for treating tumoral, infectious and/or auto-immune diseases.

CLAIMS AS AMENDED SEPTEMBER 2, 1996:

1. A conjugate consisting of an active substance and a native protein which is not regarded as exogenous, a linker cleavable in a cell being present between the active substance and the protein, characterized in that the linker comprises an azo group.
2. The conjugate according to claim 1, characterized in that the active substance is a compound usable for treating tumoral, infectious and/or autoimmune diseases.
3. The conjugate according to claim 2, characterized in that the active substance is a chemotherapeutic agent and/or a photoactive compound.
4. The conjugate according to claim 3, characterized in that the chemotherapeutic agent is an antibiotic.
5. The conjugate according to claim 3, characterized in that the chemotherapeutic agent is an antimetabolite.
6. The conjugate according to any one of claims 1 to 5, characterized in that several active substances are present.
7. The conjugate according to any one of claims 1 to 6, characterized in that the linker has the following structure:
$$-Y-R-N=N-$$
wherein:
R is an aromatic compound, and
Y is a group selected from C(O), S(O)₂, P(O)OH and As(O)OH.
8. The conjugate according to any one of claims 1 to 7, characterized in that the protein is albumin.

9. The conjugate according to claim 1, namely the conjugate of FIGURE 1.
10. The conjugate according to claim 1, namely the conjugate of FIGURE 2.
11. The conjugate according to claim 1, namely the conjugate of FIGURE 3.
12. A process for the preparation of the conjugate according to claim 1, characterized in that the binding of the active substance via the linker to the protein comprises a diazotization of amino groups and/or an activation of acid groups.
13. Use of the conjugate according to any one of claims 1 to 11 for treating tumoral, infectious and/or auto-immune diseases.

ABSTRACT

This invention relates to a conjugate which consists of an active substance and a native protein which is not regarded as exogenous and distinguishes itself in that an intracellularly cleavable linker is present between the active substance and the protein.

In addition, this invention concerns a process for the preparation of such a conjugate and its use.

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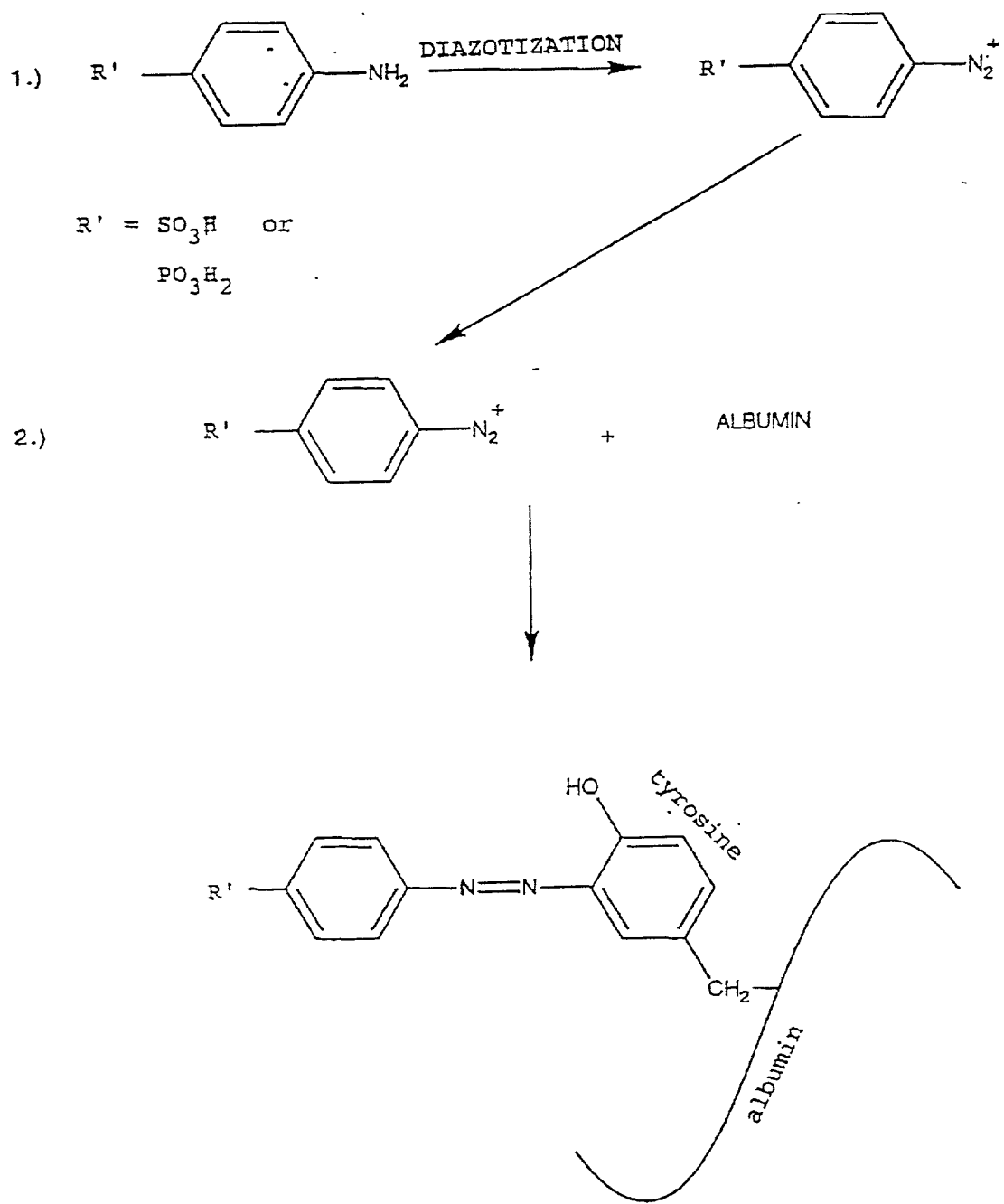


FIGURE 1

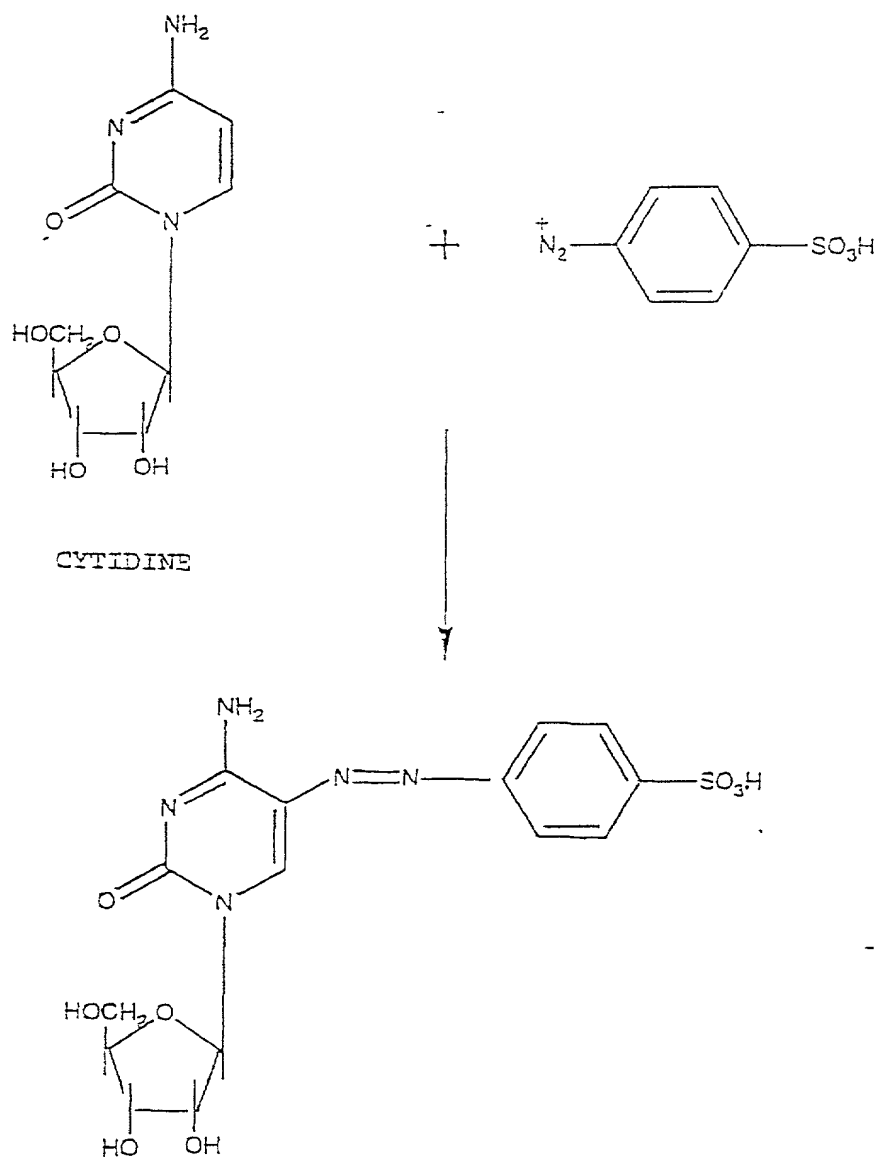
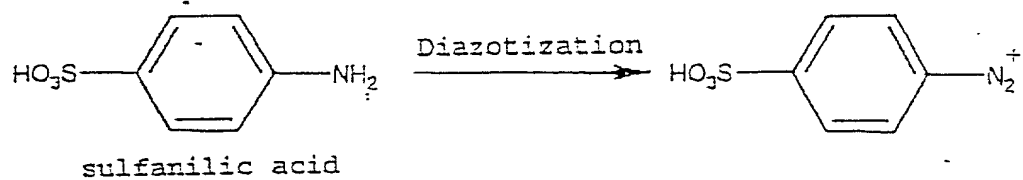
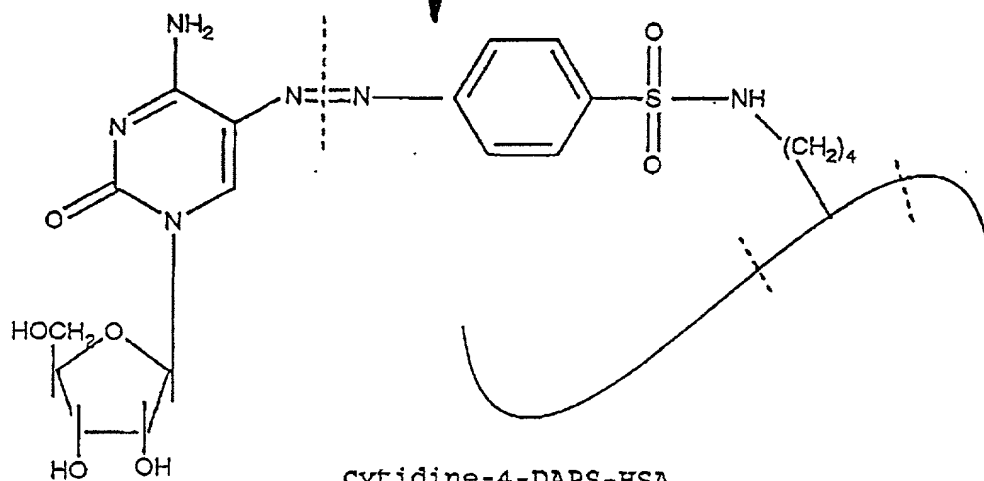


FIGURE 2A

1.+DCC and HSI

2.+ALBUMIN



cytidine-4-DAPS-HSA
(dashed lines: enzymatic cleavage)

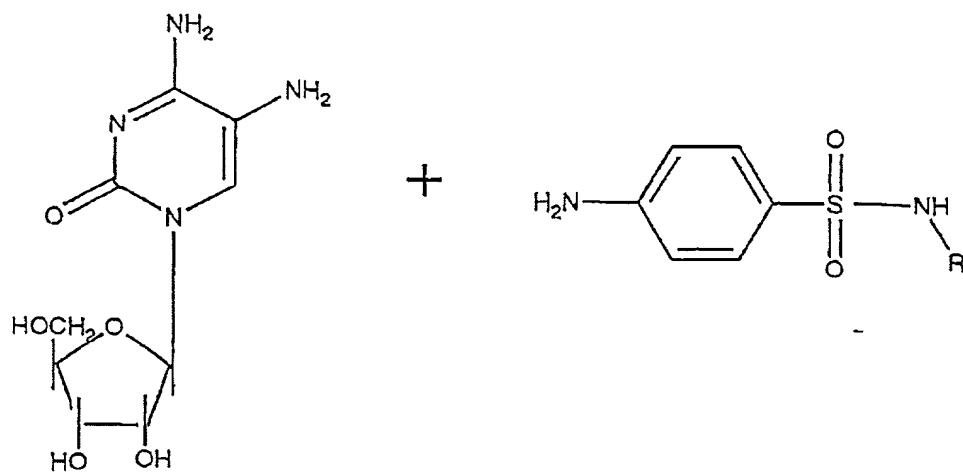
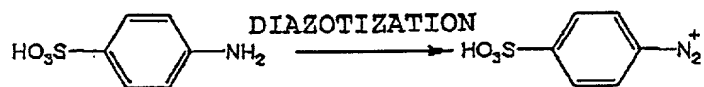


FIGURE 2B



$\text{R}'' = \text{H} \text{ or } \text{Cl}$

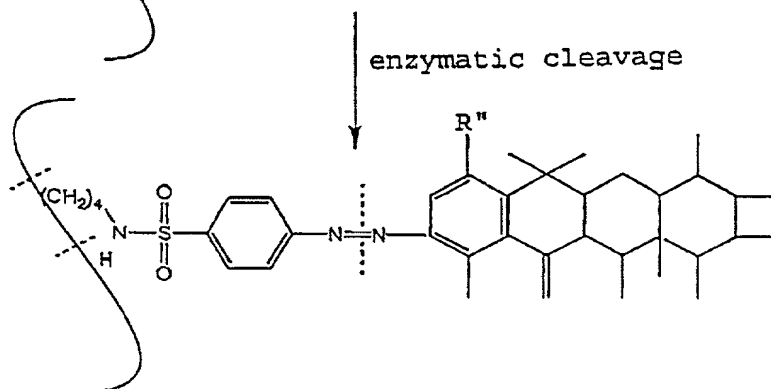
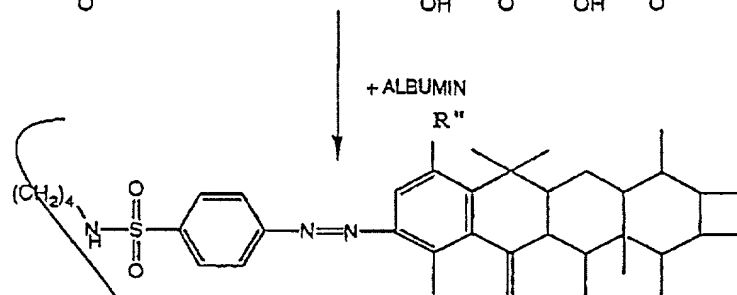
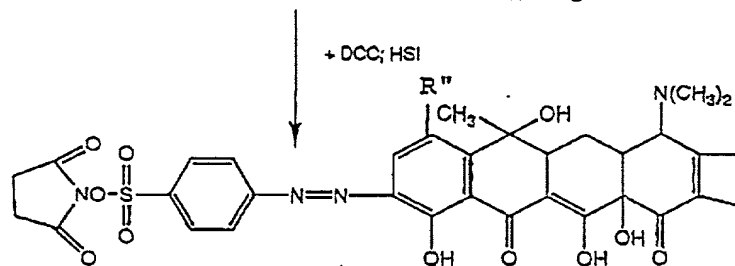
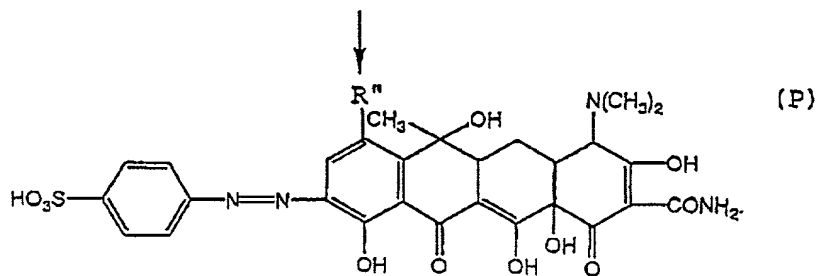
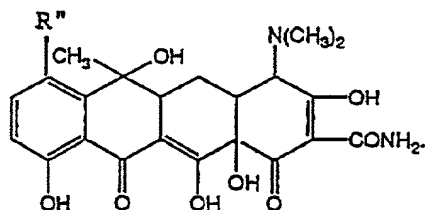
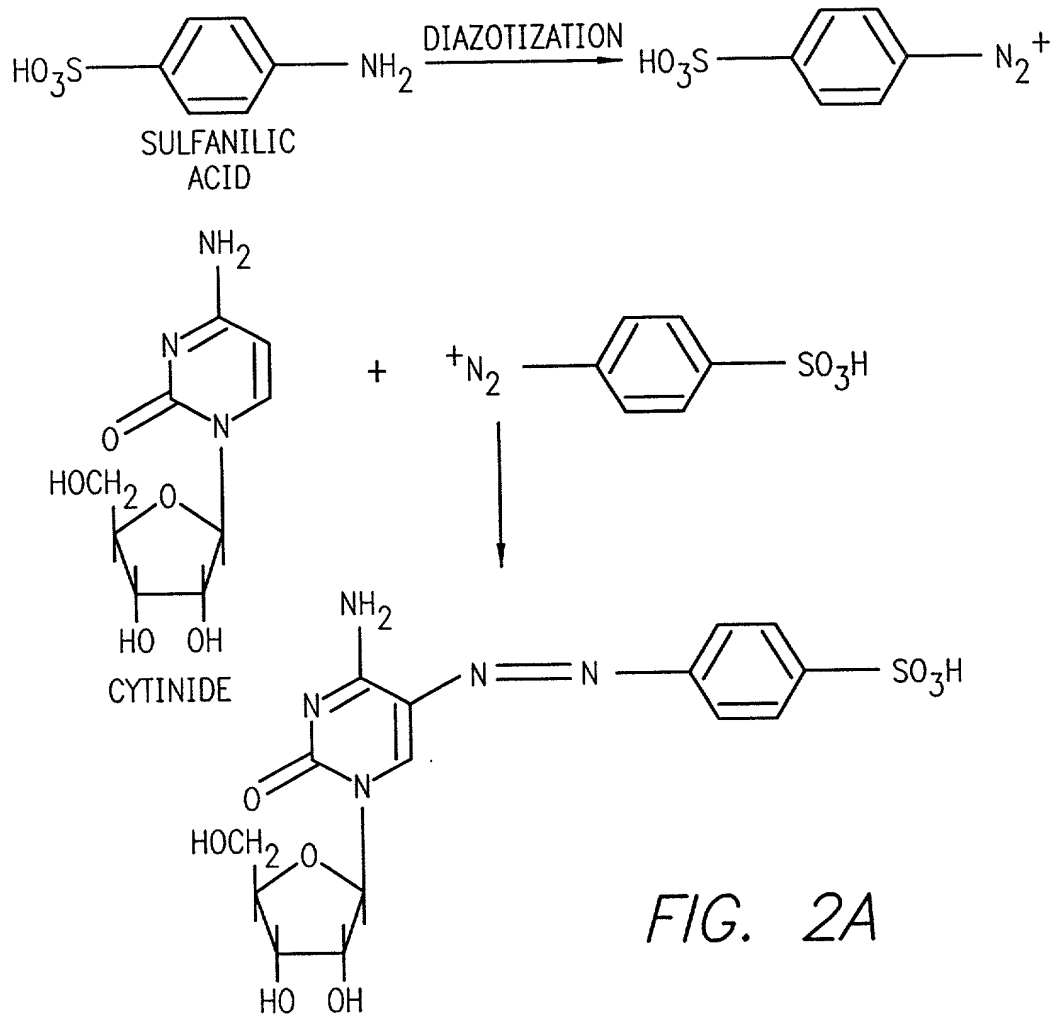
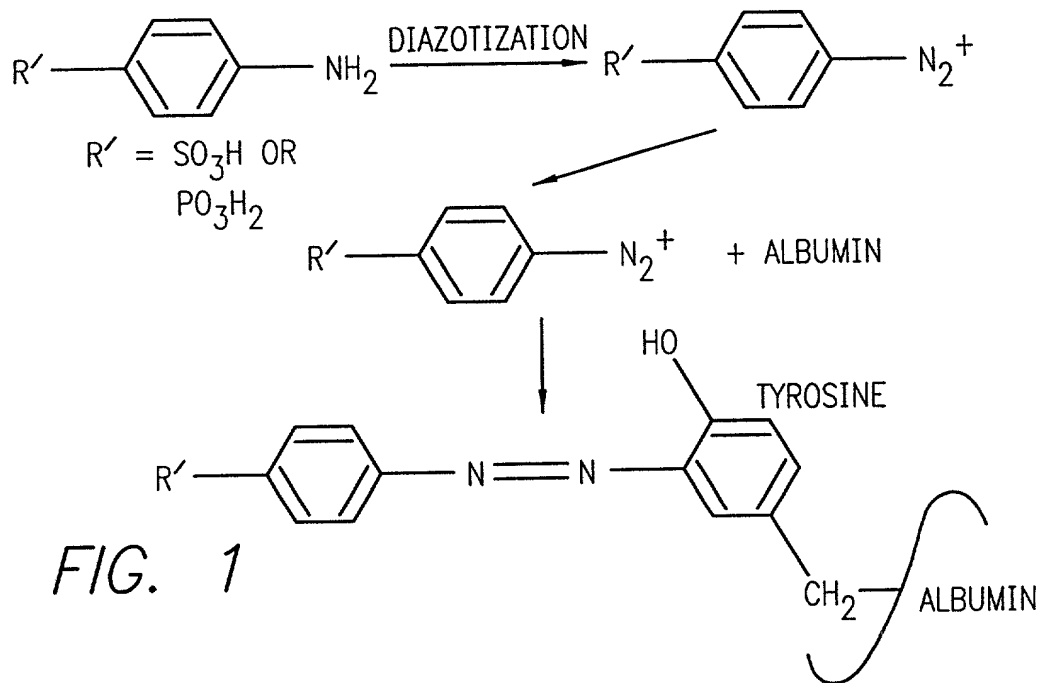


FIGURE 3

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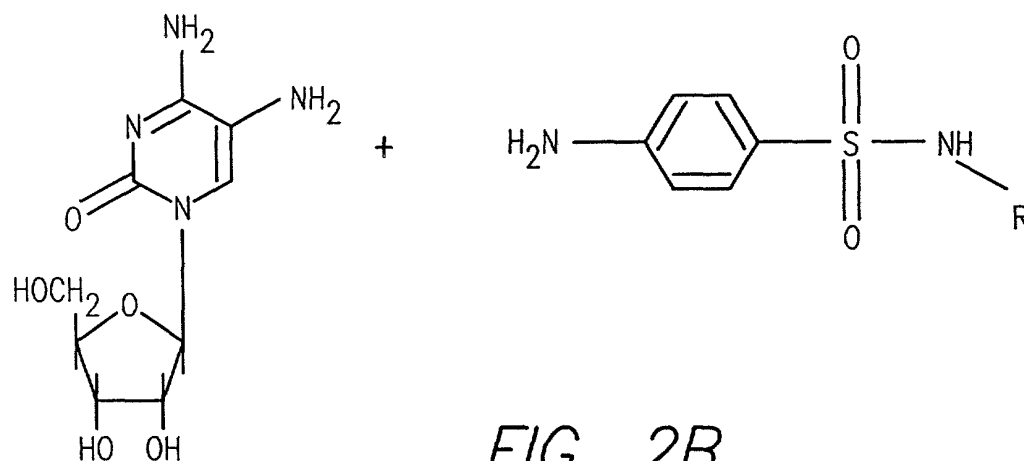
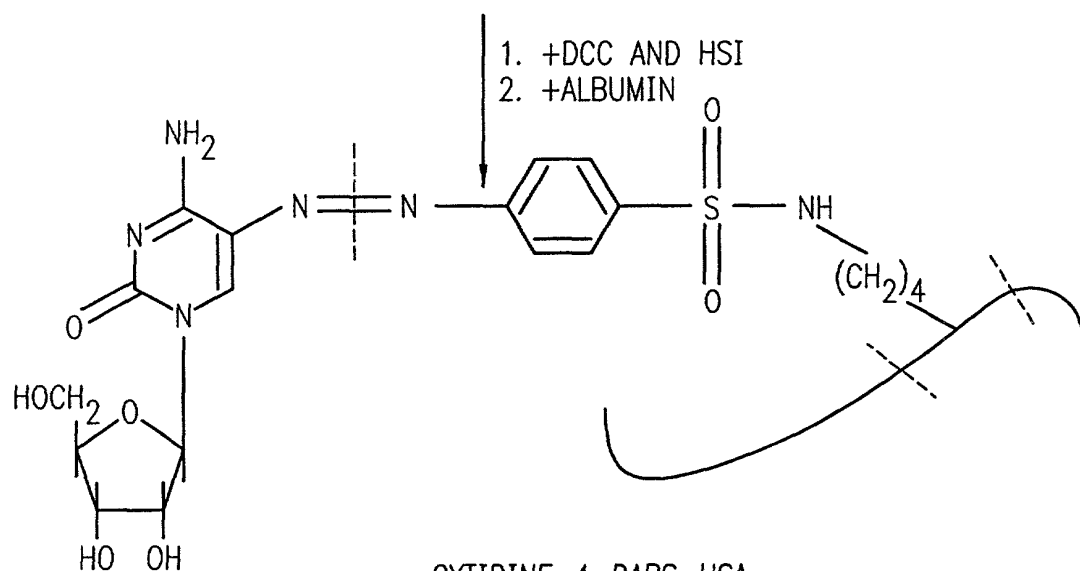


FIG. 3

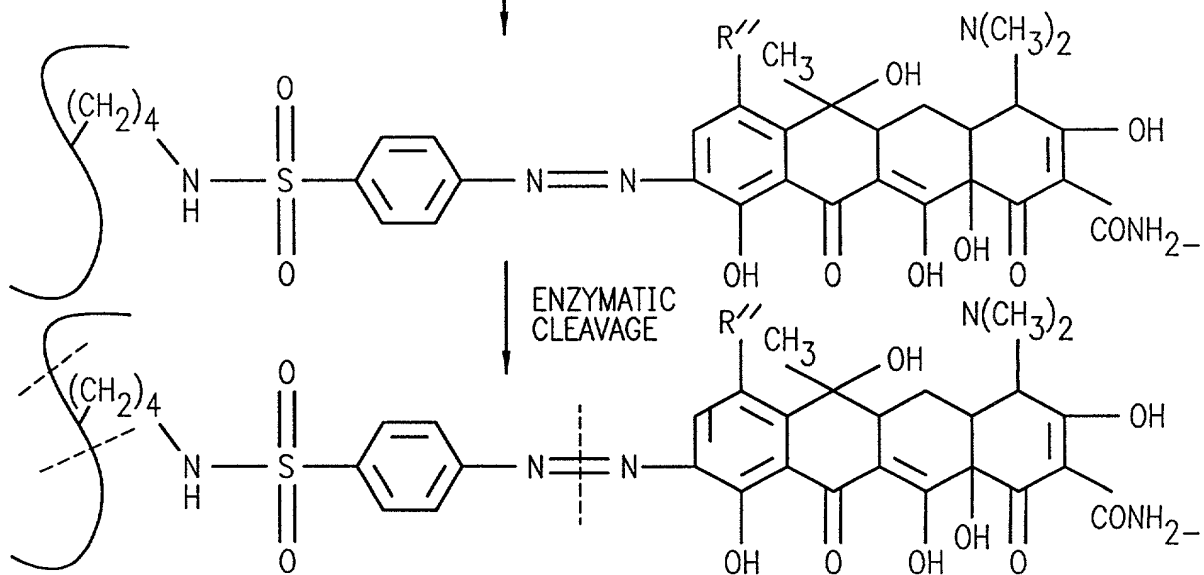
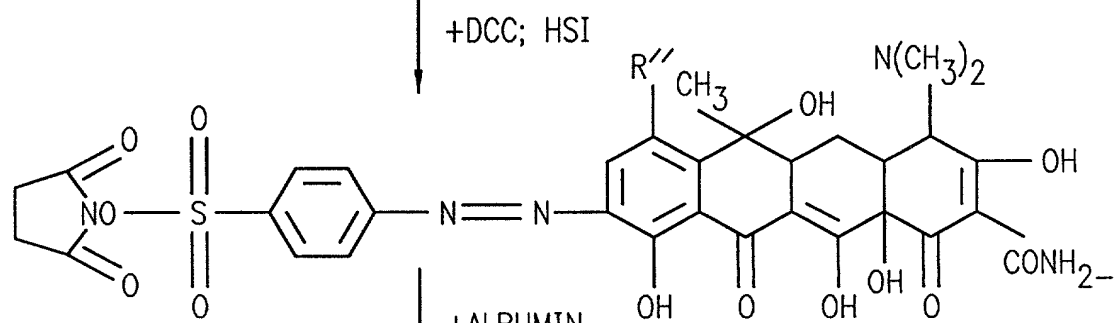
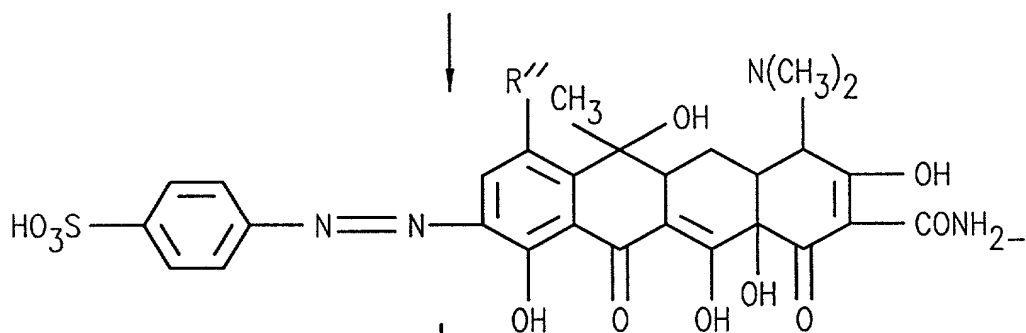
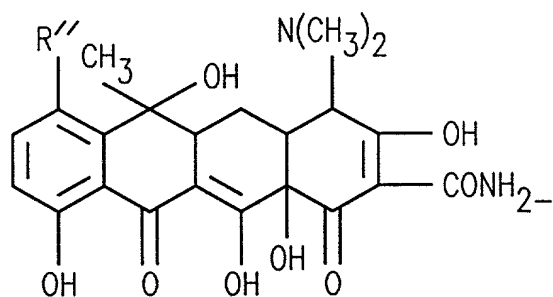
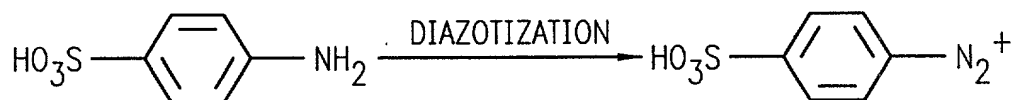


FIG. 4

